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Butyrate differentially regulates cytokines and proliferation in porcine peripheral blood mononuclear cells

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Abstract

Although butyrate modulates proliferation and cytokine production by PBMC in some species, the role of butyrate as a regulator of immunocyte function in the pig has not been studied. Therefore, the primary objective of this study was to determine whether butyrate influences peripheral blood mononuclear cell (PBMC) proliferation, cytokine secretion and mRNA expression in the pig in vitro. We also sought to determine whether alterations in cytokine production attributable to butyrate were associated with changes in the expression of suppressor of cytokine signaling-3 (SOCS3). Porcine PBMC were isolated from venous blood and stimulated with concanavalin A (ConA) in the presence or absence of sodium butyrate at 0.2 or 2.0 mM. Butyrate at 2.0 mM suppressed ($P < 0.05$) ConA-induced PBMC proliferation and led to a paradoxical increase ($P < 0.05$) in IL-2 mRNA expression. The secretion and mRNA expression of interferon-γ (IFN-γ) by ConA-activated PBMC was increased ($P < 0.05$) by butyrate at 2.0 mM. Exposing activated PBMC to butyrate at 2.0 mM decreased ($P < 0.05$) the secretion of interleukin-10 (IL-10). In contrast, butyrate at 0.2 mM increased ($P < 0.05$) both IL-10 secretion and mRNA expression. Activation of porcine PBMC with ConA increased ($P < 0.05$) the expression of SOCS3 mRNA, and butyrate treatment further augmented ($P < 0.05$) SOCS3 mRNA expression in a dose-dependent manner. Mechanistically, pretreatment with the adenyl cyclase inhibitor 2,5-dideoxyadenosine abolished ($P < 0.05$) the inhibitory effect of 2.0 mM butyrate on IL-10 secretion, and partially reversed ($P < 0.05$) the increase in IFN-γ secretion induced by 2.0 mM butyrate. These data indicate that the effect of butyrate on cytokine production by porcine PBMC is dose-dependent, and that butyrate increases the expression of SOCS3 in activated PBMC. In addition, we provide evidence that the effects of butyrate on IFN-γ and IL-10 production are mediated in part via a cAMP-dependent mechanism.

Keywords: Pigs; Butyrate; Cytokine; Suppressor of cytokine signaling

1. Introduction

Butyrate, a short-chain fatty acid produced by the bacterial fermentation of dietary fiber, has been found to profoundly impact the immune system of humans

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and rodents. Studies have found that butyrate decreases in vitro lymphocyte proliferation in mice (Kyner et al., 1976) and in rats (Cavaglieri et al., 2003). In addition, butyrate has been found to directly skew cytokine secretion to that of a T helper type 2 milieu. More specifically, butyrate increases the secretion of IL-10 (Nancey et al., 2002; Saemann et al., 2000), and decreases the secretion of IFN-γ (Nancey et al., 2002) by activated human lymphocytes in vitro. In agreement with the shift to a T helper type 2 (Th2) milieu, butyrate decreased the ex vivo production of inflammatory cytokines from intestinal biopsies of humans suffering from Chron's disease, and reduced the severity of TNBS-induced colitis in rats (Segain et al., 2000).

The mechanisms of butyrate-induced alterations in immune system function are of considerable interest. One mechanism via which butyrate may skew the immune response is through increasing the expression of suppressor of cytokine signaling (SOCS3). The SOCS proteins are selectively induced by a variety of stimuli and inhibit cytokine signaling by blocking the activation of the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway (Elliot and Johnston, 2004). The level of SOCS3 expression in Th2 cells is several fold higher than in Th1 cells (Egwuagu et al., 2002) suggesting a role for SOCS3 in T-cell polarization. Another mechanism by which butyrate could impact lymphocyte function is through a cAMP-dependent pathway. Butyrate has been found to regulate gene expression in a cAMP-dependent manner in chromaffin cells (DeCastro et al., 2005), however it is not known whether butyrate alters the function of lymphocytes via a cAMP-dependent pathway.

To date, there are no published data as to the effects of butyrate on immunological variables in the pig. Therefore, the objective of the current study was to determine whether butyrate could directly alter proliferation, cytokine gene expression, and cytokine secretion by porcine PBMC. Moreover, we also wanted to determine whether alterations in cytokine secretion and expression in response to butyrate in stimulated lymphocytes were associated with changes in SOCS3 mRNA expression. Lastly, we sought to determine whether butyrate affects cytokine secretion through a cAMP-dependent signaling pathway.

2. Materials and methods

2.1. Animals, PBMC isolation and culture

All animal care and handling procedures were approved by the Iowa State University Animal Care and Use Committee. Blood was collected from the jugular vein of healthy 3–5-month-old crossbred barrows into vacutainers containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ). The blood was centrifuged, and the buffy coats were collected and mixed with Hanks' balanced salt solution (Mediatech, Herndon, VA). The PBMC were isolated from the buffy coat plus HBSS mixture using Histopaque®-1077 (Sigma Chemical Co., St. Louis, MO). The PBMC were washed twice by centrifugation in HBSS, and the residual erythrocytes were lysed in a buffer containing 0.83% ammonium chloride (Sigma). PBMC were then stained with trypan blue (Mediatech) to assess viability, enumerated with a hemocytometer, and diluted in complete medium (RPMI-1640 (Mediatech), fetal bovine serum (FBS) (10%), L-glutamine (2 mM), HEPES (25 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml)). The viability of the PBMC utilized for these experiments exceeded 95% as assessed by the exclusion of trypan blue.

For the in vitro cytokine secretion and mRNA expression experiments, PBMC were cultured at a concentration of 4×10^6 in a total volume of 3 ml of complete medium in six-well tissue culture plates (Greiner Bio-One, Longwood, FL). Treatments consisted of basal complete medium, ConA (5 μg/ml; Sigma), ConA and sodium butyrate (Sigma) at a concentration of 0.2 or 2.0 mM. In experiments in which 2,5-dideoxyadenosine (ddA, Calbiochem, La Jolla, CA) was used, the PBMC were pretreated with 100 μM ddA for 30 min prior to the addition of the other treatments. The PBMC cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2. Supernatants were collected at 24 h after treatment for analysis of cytokine secretion into the medium, and the PBMC were harvested at 12 and 24 h post-treatment for cytokine mRNA analysis. The supernatants were stored at −80 °C until analysis, and the PBMC were resuspended in Tri® Reagent (Sigma) and stored at −80 °C pending RNA isolation.

2.2. In vitro lymphocyte proliferation assay

The in vitro proliferation of PBMC was measured using a commercially available nonradioactive cell proliferation ELISA kit (Roche Diagnostics, Penzberg, Germany). Briefly, 100 μl of PBMC, at a density of 4×10^6 cells/ml in complete medium was added to each well of a 96-well tissue culture plate (Nunc, Roskilde, Denmark). To the PBMC, 100 μl of the respective treatments was added in triplicate. The PBMC cultures were incubated for 42 h at 37 °C and 5% CO_2, after which 20 μl of 100 μM 5-bromo-deoxyuridine (BrdU) were added to each of the wells for the final 18 h of culture. To detect incorporated BrdU, the labeling medium was removed, and the cells were dried at 60 °C for 1 h. The cells were then treated for 30 min with 200 μl/well of the fixative and denaturing reagent included with the kit. The anti-BrdU-POD working solution was added to the fixed cells for 90 min, after which the cells were washed three times with 300 μl/well of the washing solution included with the kit. Substrate solution (100 μl/well) was added to the wells, and the color was allowed to develop for 30 min at room temperature. Plates were read using a microplate reader (Biotek Instruments, Winooski, VT) at a wavelength of 340 nm.

2.3. Media ELISA for IFN-γ and IL-10 secretion

The quantity of IFN-γ in the supernatants was measured using a commercially available ELISA kit specific for porcine IFN-γ (Pierce Endogen, Rockford, IL). The IFN-γ ELISA has a sensitivity of less than 2.0 pg/ml and a range of 8.0–500 pg/ml. Supernatant IL-10 concentrations were determined with a porcine specific IL-10 ELISA kit (R&D Systems, Minneapolis, MN) with a range of 31.2–2000 pg/ml. The ELISA were conducted according to the manufacturer's recommendations.

2.4. Real-time RT-PCR for cytokines

The PBMC were thawed, and total RNA was isolated using Tri® Reagent (Sigma) according to the manufacturer's protocol, and the RNA pellets were resuspended in nuclease free water. To eliminate possible genomic DNA contamination, the RNA samples were treated with a DNase I kit (DNA-free, Ambion, Inc., Austin, TX) per the manufacturer's instructions. The total RNA was quantified by measuring the absorbance at 260 nm using a SmartSpec™ 3000 spectrophotometer (BioRad Laboratories, Hercules, CA), and the purity was assessed by determining the ratio of the absorbance at 260 and 280 nm. All samples had 260/280 nm ratios above 1.8. Additionally, the integrity of the RNA preparations was verified by visualization of the 18 and 28S ribosomal bands stained with ethidium bromide after electrophoresis on 1.2% agarose gels. Total RNA (1 μg) was reverse transcribed using a commercially available cDNA synthesis kit (iScript, BioRad).

Real-time PCR detection of cytokine mRNAs was conducted utilizing the SYBR Green assay. Primers used for real-time PCR are presented in Table 1. Amplification was carried out in a total volume of 25 μl containing 1× iQ™ SYBR® Green Supermix (BioRad), forward and reverse primers (0.1 μg/μl) and 1 μl of the cDNA reaction. After an initial 5 min denaturation step at 95 °C, the reactions were cycled 40 times under the following parameters: 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s. Optical

Table 1
Primers used for cytokine mRNA in porcine PBMC

Gene	Primer sequences[a]	Accession number
IFN-γ	(S) CAGAGCCAAATTGTCTCCTTCTAC, (AS) TCTCTGGCCTTGGAACATAG	NM_213948
IL-10	(S) GCATCCACTTCCCAACCA, (AS) CTTCCTCATCTTCATCGTCAT	NM_214041
IL-2	(S) GATTTACAGTTGCTTTTGAAG, (AS) GTTGAGTAGATGCTTTGACA	NM_213861
IL-4	(S) GTCTCACATCGTCAGTGC, (AS) TCATGCACAGAACAGGTC	L12991
SOCS3[b]	(S) AGATCCCTCTGGTGTTGAGC, (AS) CGTTGACTGTTTTCCGACAG	AY785557
β-Actin	(S) GGACCTGACCGACTACCTCA, (AS) GCGACGTAGCAGAGCTTCTC	U07786

[a] S: sense primer, AS: antisense primer.
[b] Suppressor of cytokine signaling-3.

detection was carried out at 72 °C. At the end of the PCR, melt curve analysis was conducted to validate the specificity of the primers. Thermal cycling conditions and real-time detection were conducted using an Opticon real-time PCR detection system (BioRad). A nontemplate control was run with every assay, and all determinations were performed in duplicates. External cDNA standards were constructed by cloning the corresponding RT-PCR product into a pCR® 4-TOPO® vector (Invitrogen, Carlsbad, CA), and the resultant plasmids were sequenced for verification. The abundance of each gene product was calculated by regressing against the standard curve generated in the same reaction with their respective plasmids. The RNA abundance values for each sample were normalized to β-actin.

2.5. Statistical analysis

The statistical analyses were conducted as ANOVAs using the GLM procedures of Statistical Analysis System Version 8.0 software (SAS Institute, Inc., Cary, NC). When ANOVA indicated a significant difference, means within each time point were separated using the PDIFF and STDERR options in GLM. Pig served as the experimental unit. The data were subjected to log transformation prior to analysis to obtain homogeneity of variance. Data are presented as non-transformed mean ± S.E. A significance level of $P < 0.05$ was used.

3. Results

3.1. In vitro PBMC proliferation

The effect of butyrate on in vitro ConA-induced proliferation of porcine PBMC is presented in Fig. 1. The addition of sodium butyrate at 0.2 mM had no effect on ConA-induced proliferation. However, when the concentration of sodium butyrate was increased to 2.0 mM the ConA-induced proliferative response was reduced ($P < 0.05$) by 73%.

3.2. Cytokine secretion and expression

As expected, exposing the PBMC to ConA-induced ($P < 0.05$) the secretion of IFN-γ into the media (Fig. 2A). Sodium butyrate at a concentration of 2.0 mM, but not 0.2 mM, further augmented ($P < 0.05$) the ConA-induced secretion of IFN-γ by PBMC. As found with IFN-γ, ConA increased

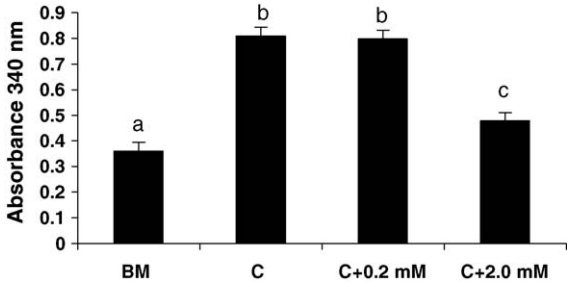

Fig. 1. Effect of butyrate on in vitro porcine PBMC proliferation. The PBMC were incubated in the presence of the specific treatments for 42 h, after which 100 μM 5-bromo-deoxyuridine (BrdU) was added to the cultures for the final 18 h. Treatments included basal medium (BM), ConA at 5 μg/ml (C), ConA and sodium butyrate at 0.2 mM (C + 0.2 mM), or ConA and sodium butyrate at 2.0 mM (C + 2.0 mM). $n = 6$ pigs. Treatments that do not have common letters differ ($P < 0.05$).

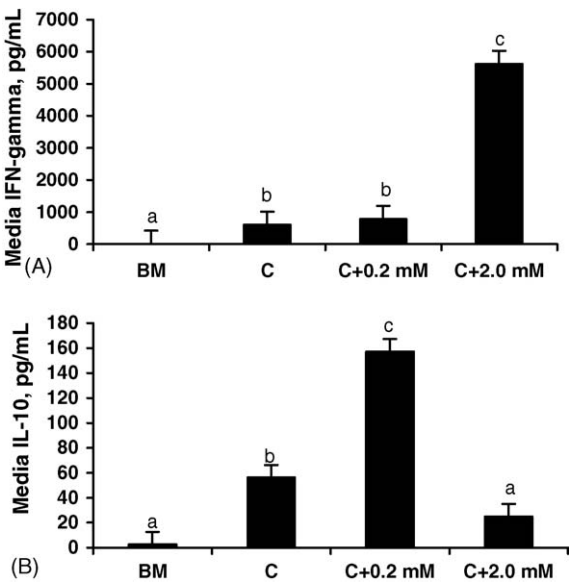

Fig. 2. Effect of butyrate on mitogen induced (A) IFN-γ and (B) IL-10 secretion by PBMC, in vitro. The PBMC were incubated in the presence of the treatments for 24 h. The treatments included basal medium (BM), ConA at 5 μg/ml (C), ConA and sodium butyrate at 0.2 mM (C + 0.2 mM), or ConA and sodium butyrate at 2.0 mM (C + 2.0 mM). $n = 6$ pigs. Treatments that do not have common letters differ ($P < 0.05$).

($P < 0.05$) the secretion of IL-10 by PBMC (Fig. 2B). However, there was a dose-dependent response in terms of IL-10 secretion when sodium butyrate was added to the stimulated PBMC. Sodium butyrate at a concentration of 0.2 mM increased ($P < 0.05$) ConA-induced IL-10 secretion, whereas 2.0 mM sodium butyrate inhibited ($P < 0.05$) IL-10 secretion by PBMC.

In agreement with the IFN-γ secretion data, treatment of the PBMC with ConA increased ($P < 0.05$) the expression of IFN-γ mRNA (Fig. 3A). Treating the stimulated PBMC with 2.0 mM sodium butyrate further increased ($P < 0.05$) the relative abundance of IFN-γ mRNA at both 12 and 24 h after stimulation. The expression of IL-10 mRNA was increased ($P < 0.05$) by ConA at 12 and 24 h (Fig. 3B). An enhancement ($P < 0.05$) of ConA-induced IL-10 expression was found at 12 h in stimulated PBMC treated with 0.2 mM sodium butyrate. At 24 h after stimulation, the effect of sodium butyrate on IL-10 mRNA in stimulated PBMC was not evident. Increased ($P < 0.05$) levels of IL-2 mRNA were found in ConA-stimulated PBMC at 12 and 24 h after initiating the treatments (Fig. 2C). Adding 2.0 mM sodium butyrate to PBMC stimulated with ConA further increased ($P < 0.05$) the expression of IL-2 mRNA at 12 h. Treating the PBMC with 0.2 mM sodium butyrate led to levels of IL-2 mRNA that were between that found in unstimulated and stimulated PBMC at 24 h after stimulation. While the magnitude of IL-4 mRNA expression was several fold lower than the other cytokines measured, ConA was still found to increase ($P < 0.05$) the relative abundance of IL-4 mRNA at 12 h after treatment (Fig. 2D). Adding sodium butyrate at a concentration of 2.0 mM decreased the expression of IL-4 mRNA to the level of control cultures at 12 h after treating the PBMC. There was no effect of treatment on the relative abundance of IL-4 mRNA at the 24 h time point.

3.3. Suppressor of cytokine-3 (SOCS3) expression and the effect of 2,5-dideoxyadenosine (ddA)

Stimulating porcine PBMC with ConA increased ($P < 0.05$) the relative abundance of SOCS3 mRNA at 12 h after treatment (Fig. 4). However, the expression

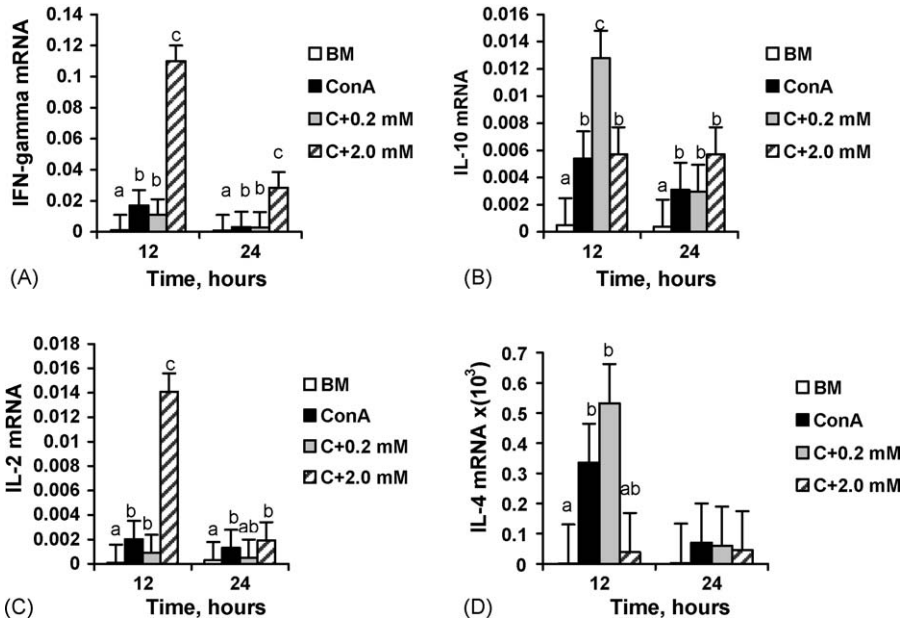

Fig. 3. Effect of in vitro treatment with ConA and butyrate on (A) IFN-γ, (B) IL-10, (C) IL-2, and (D) IL-4 mRNA relative abundance in porcine PBMC. The cytokines are normalized to the expression of β-actin. The PBMC were incubated in the presence of the treatments for 12 or 24 h. Treatments included basal medium (BM), ConA at 5 μg/ml (C), ConA and sodium butyrate at 0.2 mM (C + 0.2 mM), or ConA and sodium butyrate at 2.0 mM (C + 2.0 mM). $n = 6$ pigs. Treatments within a time point that do not have common letters are different ($P < 0.05$).

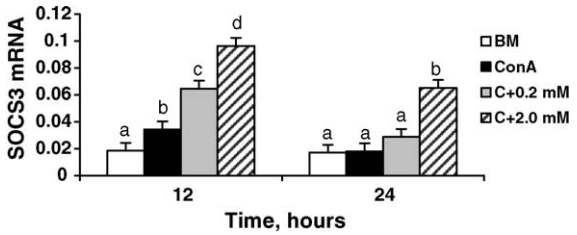

Fig. 4. Effect of ConA and butyrate on the relative abundance of suppressor of cytokine signaling-3 (SOCS3) mRNA in porcine PBMC. The PBMC were incubated in the presence of the treatments for 12 or 24 h. Treatments included basal medium (BM), ConA at 5 μg/ml (C), ConA and sodium butyrate at 0.2 mM (C + 0.2 mM), or ConA and sodium butyrate at 2.0 mM (C + 2.0 mM). $n = 6$ pigs. Treatments within a time point that do not have common letters differ ($P < 0.05$).

of SOCS3 was not increased in ConA-stimulated PBMC at 24 h. Treating stimulated PBMC with sodium butyrate led to a dose-dependent increase ($P < 0.05$) in the relative abundance of SOCS3 mRNA. The level of expression of SOCS3 remained elevated ($P < 0.05$) at 24 h in ConA-stimulated PBMC that were cultured in the presence of 2.0 mM sodium butyrate.

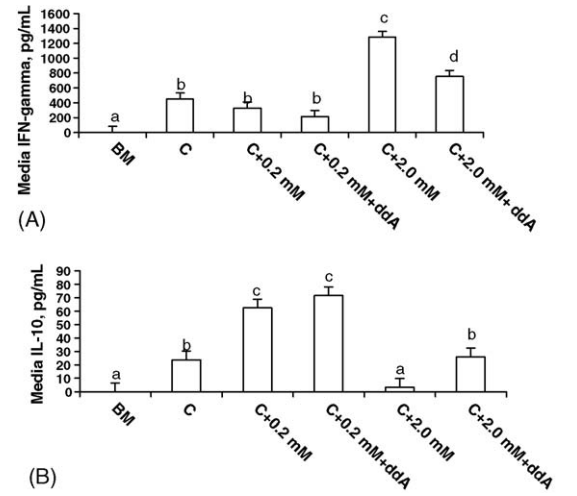

Fig. 5. Effect of adenylate cyclase inhibition on butyrate induced alterations on in vitro (A) IFN-γ and (B) IL-10 secretion by porcine PBMC. The PBMC were incubated in the presence of the treatments for 24 h. Treatments included basal medium (BM), ConA at 5 μg/ml (C), ConA and sodium butyrate at 0.2 mM (C + 0.2 mM), or ConA and sodium butyrate at 2.0 mM (C + 2.0 mM). For the treatments in which 2,5-dideoxyadenosine (ddA) was included, the cells were pretreated with 100 μM ddA for 30 min before the addition of the other treatments. $n = 3$ pigs. Treatments that do not have common letters differ ($P < 0.05$).

As expected from the results of the earlier experiments, sodium butyrate dose-dependently regulated the secretion of IFN-γ and IL-10 from ConA-stimulated PBMC (Fig. 5). Treating stimulated PBMC with 0.2 mM sodium butyrate increased ($P < 0.05$) the secretion of IL-10. The secretion of IFN-γ was increased ($P < 0.05$) and the secretion of IL-10 was decreased ($P < 0.05$) in stimulated PBMC treated with 2.0 mM sodium butyrate. Pretreating the PBMC with 100 μM ddA completely reversed ($P < 0.05$) the inhibitory effect of 2.0 mM sodium butyrate on IL-10 secretion, and partially reversed ($P < 0.05$) the augmentation of IFN-γ secretion by 2.0 mM sodium butyrate. However, treating with ddA had no effect on the increased secretion of IL-10 found in PBMC treated with 0.2 mM sodium butyrate.

4. Discussion

In this report we provide initial evidence that butyrate regulates the immune response in the domestic pig and that the effect of butyrate is somewhat different from what has been observed in other species. As found in human (Cavaglieri et al., 2003) and rodent models (Kyner et al., 1976), treatment of porcine PBMC with butyrate led to a dose-dependent decrease in ConA-induced proliferation. However, in porcine PBMC butyrate differentially skewed the cytokine profile to that of a Th1 or Th2 depending on the concentration of butyrate used. Treating porcine PBMC with the lower concentration (0.2 mM) of butyrate increased the mRNA expression and secretion of IL-10, but had no effect on IFN-γ secretion or mRNA abundance. The increase in IL-10 and lack of an effect on IFN-γ is in agreement with what has been found in human lymphocytes treated with a similar dose of butyrate (Nancey et al., 2002; Saemann et al., 2000). Increasing the concentration of butyrate to 2.0 mM led to an increased secretion and mRNA expression of IFN-γ and decreased secretion of IL-10 in porcine PBMC. Additionally, the expression of IL-2 mRNA was increased and the expression of IL-4 mRNA was decreased. This finding is in contrast to what has been observed in humans (Nancey et al., 2002) and rats (Cavaglieri et al., 2003) where

butyrate at higher concentrations (1–2 mM) inhibits IFN-γ secretion and enhances IL-10 production by ConA-activated lymphocytes. These findings indicate that in the pig butyrate at higher concentrations regulates cytokine production differently to that found in rodents and humans.

It is interesting that butyrate at the higher dose tested transiently increased the expression of IL-2 mRNA yet decreased the proliferation of porcine PBMC. While we did not measure IL-2 protein secretion by the porcine PBMC, it is highly unlikely that the increase in IL-2 mRNA expression did not translate into an increased amount of secreted IL-2. The results of previous research indicate a strong relationship between IL-2 mRNA and IL-2 protein secretion in ConA-stimulated porcine PBMC (Verfaillie et al., 2001). It has been observed that butyric acid and butyric acid derivatives inactivate antigen stimulated T-cells (Soderberg et al., 2004) and induce anergy in Th1 cells (Jackson et al., 2002). Furthermore, a related metabolite *n*-butyrate has been shown to inhibit the activation of the nuclear factor of activated T-cells (NF-AT) in activated Jurkat T-cells leading to a decrease in IL-2 transcription (Diakos et al., 2002). This indicates that porcine PBMC respond differently to butyrate in terms of IL-2 expression. Research conducted with human PBMC has found that butyrate treatment dose-dependently induces apoptosis (Sourlingas et al., 2001). The induction of apoptosis, along with the induction of anergy may be mechanisms via which higher doses of butyrate ultimately decrease PBMC proliferation.

The in vitro model used in our experiments may not be totally representative of events occurring at the intestinal level where butyrate levels are similar or greater than our higher (2.0 mM) treatment concentration (Biagi et al., 2006; Wuerthrich et al., 1998), but the results warrant further investigation into cytokine regulation by butyrate in the pig. It is of interest to note that adding certain fiber sources to the diet has been found to increase plasma butyrate concentrations in rats (Storer et al., 1983) and pigs (Knudsen et al., 2005) to levels approaching the lower concentration (0.2 mM) of butyrate used in our studies. Furthermore, feeding fiber sources that elevate circulating butyrate levels may decrease the magnitude of the inflammatory process via increasing IL-10 production by activated lymphocytes. Interleukin-10 is a prototypical Th2 cytokine that inhibits the expression of proinflammatory cytokines (Del Prete et al., 1993; Fiorentino et al., 1991) and is believed to reverse the activation of macrophages (Ma et al., 2003).

The biological significance of increased IFN-γ by porcine PBMC in response to elevated concentrations of butyrate remains to be determined. It is highly unlikely that in response to dietary factors blood butyrate concentrations in pigs ever reach the magnitude of the higher dose of butyrate used in our experiment (Knudsen et al., 2003). An elevation in IFN-γ in response to concentrations of butyrate found in the intestinal tract is in contrast to the anti-inflammatory properties of butyrate observed in other species. Butyrate has been found to decrease the activation of macrophages from patients with ulcerative colitis (Luhrs et al., 2002) and decrease the production of proinflammatory cytokines from intestinal tissue collected from Chron's disease patients (Segain et al., 2000). In addition, supplementing the diet with a fiber source that increases intestinal butyrate levels is associated with anti-inflammatory properties in a rodent model of gastrointestinal inflammation (Rodriguez-Cabezas et al., 2003). Furthermore, any elevations of IFN-γ production may be counteracted by the ability of butyrate to inhibit IFN-γ induced macrophage activation, as has been observed in microglia cells (Kim et al., 2004).

Treating the porcine PBMC with ConA increased the expression of SOCS3 and culturing activated PBMC in the presence of butyrate further increased the expression of SOCS3 mRNA in a dose-dependent fashion. In preliminary experiments, we observed no effect of butyrate on SOCS3 mRNA in resting PBMC (data not shown). Furthermore, this is the first report of butyrate regulating the expression of SOCS3 in any cell type, and may partially explain the anti-inflammatory effects observed with butyrate. The increase in SOCS3 by butyrate may be physiologically relevant at the intestinal level as the overexpression of SOCS3 has been shown to inhibit the activation of the transcription factor NFκB by IL-6 in intestinal cells in vitro (Wang et al., 2003). Our finding that butyrate at the lower concentration (0.2 mM) increased SOCS3 mRNA expression suggests a novel means for butyrate to regulate inflammation outside of the intestinal tract. Increasing SOCS3 expression is associated with decreased cytokine signaling and macrophage

activation in response to various inflammatory stimuli (Dalpke et al., 2001; Jo et al., 2005). The dose-dependant increase in SOCS3 induced by butyrate was not associated with any particular Th skewing with regards to cytokine production. This is interesting given that the expression of SOCS3 has been found to be associated with Th2 polarization (Egwuagu et al., 2002; Nishiura et al., 2004).

To date there is little information as to the signaling pathway used by butyrate to alter cytokine production. Butyrate has been found to alter the expression of the tyrosine hydroxylase gene in rat chromaffin cells (PC12) via a cAMP-dependent pathway (DeCastro et al., 2005). Treating activated Jurkat T-cells with *n*-butyrate leads to an increase in intracellular cAMP levels (Diakos et al., 2002). We have extended the finding of cAMP-dependent regulation by butyrate to cytokine production by porcine PBMC. The inhibition of adenyl cyclase with ddA, and presumed decrease in cAMP, led to a complete reversal of the inhibition of IL-10 production and a partial reversal of the augmentation of IFN-γ production by the higher concentration of butyrate used in our studies. This finding indicates that cAMP is implicated in at least some of the alterations in cytokine production induced by butyrate in porcine PBMC.

In summary, butyrate differentially regulated cytokine expression and secretion by porcine PBMC in a dose-dependent manner. At a lower concentration butyrate increased the secretion of IL-10, and at a higher concentration butyrate skewed cytokine mRNA expression and secretion to a Th1 milieu. As established earlier in other species, butyrate at a higher concentration inhibited the proliferation of porcine PBMC, but led to a paradoxical increase in IL-2 mRNA expression. Butyrate, at both concentrations tested, increased the expression of SOCS3 which indicates a novel anti-inflammatory mechanism used by butyrate to inhibit cytokine signaling. The regulation of IFN-γ and IL-10 production by butyrate in porcine PBMC appears to be at least partially mediated via a cAMP-dependent pathway.

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